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Protein depletion and refeeding change the proportion of mouse liver glutathione *S*-transferase subunits

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Abstract

The effect of protein depletion followed by refeeding with a normal diet on the content of mouse liver cytosolic proteins was studied. By peptide-mass fingerprinting and N-terminal sequencing, three polypeptides whose contents changed with dietary protein level were identified as glutathione *S*-transferases (GST) Yb1, Yc and Yf subunits. Five days of depletion caused the increase of Yb1 and Yf (21.6% and 78.5%, respectively) and the decrease of Yc (31.2%). After two days of refeeding, Yb1 and Yc were practically restored, while the neoplastic marker Yf remained higher (63.4%). None of the nutritional conditions tested induced new GSTs. While protein depletion–refeeding altered the ratios between the constitutive GST subunits, total liver GST content and activity were unaffected by depletion and slightly increased by refeeding. The increased amounts of Yb1 and Yf, and the maintenance of total GST content, indicate that during protein depletion, the GST subunits levels are controlled by mechanisms different from the majority of cytosolic proteins. © 1997 Elsevier Science B.V.

Keywords: Glutathione *S*-transferase; Subunit proportion; Liver; Protein-free diet; Refeeding; (Mouse)

1. Introduction

The protein content in mouse liver decreases up to 50% upon five days of protein depletion [1]. It can be restored to normal values after one day of protein refeeding in which the protein degradation rate is practically suppressed [1]. This behavior is observed in cytosolic, nuclear, microsomal and lysosomal–mitochondrial proteins [2]. In addition, the proportion and synthesis rate of several cytosolic proteins seems to be affected by dietary protein intake. In fact, we have demonstrated that dietary protein depletion causes an increase in both the content and synthesis rate of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and of a non-identified 27 000-Da polypeptide [3]. As high levels of GAPDH have also been detected during hepatic and renal carcinogenesis [4,5], and alternated cycles of protein depletion and refeeding have been associated with liver vulnerability to carcinogenesis [6], we further investigated whether the levels of other liver cytosolic proteins change in response to dietary protein intake.

We now report that three polypeptides of 27 000, 26 000 and 25 000 Da whose content is regulated by

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dietary protein intake are glutathione *S*-transferase (E.C. 2.5.1.18) subunits (GSTs) [7]. GSTs are believed to participate in the intracellular detoxification of mutagens, carcinogens and other noxious chemical substances [8]. They have also been involved in protection mechanisms against oxidative stress and in the acquired drug resistance of cancer cells during chemotherapy [8,9]. In addition, they have been considered as useful markers for both various cancers and high-risk precancerous lesions [9]. Finally, although liver GST activity per mg of cytosolic protein has been previously estimated in protein depleted–refed mice [10], this work reports the effect of protein depletion and refeeding on GST content, activity and subunits proportions.

2. Materials and methods

2.1. Diets

Protein depletion diet was prepared as indicated in USP XV [11] except that dextrin was replaced by the same amount of sucrose; its composition expressed as percentage of dry matter was as follows: Sucrose 86%, Corn Oil 9%, Salt and Vitamin mixture 5%. The normal diet composition was as follows: Casein 23%, Sucrose 63%, Corn Oil 9%, Salt and Vitamin mixture 5%. This diet is isocaloric with respect to protein depletion diet.

2.2. Chemicals

S-hexylglutathione–agarose, 1-chloro-2,4-dinitrobenzene (CDNB) and glutathione (GSH) were obtained from Sigma Chemical, St. Louis, MO, USA. All the other reagents were of the highest purity available.

2.3. Animals and treatments

Female BALB/C mice (average body weight 24–27 g) were bred by INTA, Balcarce, Argentina. They were kept at 22°C for one week after arrival in a room illuminated from 07:00 to 19:00 h, and fed on a normal diet and water ad libitum. Protein depletion was started at 19:00 h by providing the protein depletion diet in a sufficient amount to maintain feeding ad libitum; five days later some animals were refed with a normal diet.

2.4. Enzyme assay and protein content

Glutathione *S*-transferase activity was measured at 30°C using CDNB as substrate as indicated by Habig et al. [12]. One unit of enzyme produces 1 μmol of product/min under the reaction conditions used. Protein content was measured according to Bradford [13] with bovine serum albumin as standard.

2.5. Purification of glutathione S-transferases

Four livers per nutritional condition were collected as indicated by Conde and Scornik [1] and perfused with 5 ml of cold buffer A (0.15 M NaCl, 1 mM EDTA, 5 mM β-mercaptoethanol and 20 mM Tris-HCl buffer; pH 7.4). Then they were homogenized together in 4 volumes of buffer A per gram of fresh weight. The homogenate was centrifuged at $26\,000 \times g$ for 15 min and the resulting supernatant was further centrifuged at $100\,000 \times g$ for 60 min. The supernatant was collected and assessed as crude cytosol. All operations were conducted at 4°C. The cytosol was dialyzed for 24 h against buffer A (two changes) and then applied to a *S*-hexylglutathione–agarose column (1.9 cm × 7 cm) also equilibrated with buffer A. After washing until no protein was detected in the effluent, the bound glutathione *S*-transferases were eluted with buffer B (10 mM GSH in 20 mM Tris-HCl, pH 9.6, containing 0.15 M NaCl, 1 mM EDTA and 5 mM β-mercaptoethanol). The eluate was then concentrated in a Centriflo ultrafiltration cone (AMICON) with a CF25 membrane.

The separation of glutathione *S*-transferase subunits was performed by gradient elution as follows: cytosols were applied to the *S*-hexylglutathione–agarose column (1.6 cm × 7 cm) and washed with 250 ml of buffer A. Elution of GST enzymes was developed in four stages: 0–10 mM GSH in buffer A over 200 ml; 10–50 mM GSH in buffer A over 100 ml; 50 mM GSH in buffer A over 150 ml; and 100 ml of buffer B. Fractions of 2.6 ml were collected and tested for GST activity and protein content.

2.6. HPLC analysis of glutathione S-transferases

The subunit composition of the mouse liver GSTs eluted from the affinity column was determined by reverse-phase HPLC (RP-HPLC) on a 4.6 mm × 250 mm Bio Rad C18 column by the method of Ostlund Farrants et al. [14]. Samples containing 25 μg of GSTs were injected on to the column. During 5 min, the run was isocratical and the flow rate was increased linearly from 0.5 to 1.5 ml/min. Thereafter, the flow rate was maintained and a gradient elution from 30% acetonitrile in 0.1% (v/v) trifluoroacetic acid to 55% acetonitrile in 0.1% (v/v) trifluoroacetic acid was applied over a 40-min period. The eluate was monitored at 214 nm. Individual peaks were integrated to determine sample area and then collected. For the estimation of contents, the resulting areas were compared with those displayed by known amounts of Yb1, Yc and Yf GST subunits. A linear relationship (r values > 0.985) between peak area and micrograms of subunit protein was found.

2.7. In-gel trypsin digestion and mass spectrometric analysis of tryptic peptides

Protein samples were solubilized in SDS sample buffer and run on a 16% acrylamide slab gel under reducing conditions, according to Laemmli [15]. Coomassie Blue stained protein bands were excised with a scalpel and in-gel trypsin digested as described by Rosenfeld et al. [16]. The resulting tryptic peptides were subjected to analysis on a laser MAT (Finnigan MAT, San José, CA, USA). Mass values for all the samples were the average of at least four analyses, each involving 20–50 pulses. Oxidized β-chain of bovine insulin (Boehringer Mannheim, Germany) was used for the calibration of the mass spectrometer.

2.8. Mass fingerprint analysis

The peptide masses obtained after tryptic digestion were used to search a protein database for proteins that match the experimental data using a recently developed algorithm that is available upon request [17]. Briefly this computer program, computes the molecular weight and isoelectric point (if it is known) of the protein under study and selects those entries having theoretical values within experimental ranges. Then, for these entries the program determines the set of internal peptides that would result from a tryptic digestion and computes the theoretical molecular weights of the resulting peptides. The experimental and theoretical molecular weight of the tryptic peptides are then compared and classified in a hierarchical way and protein sequences that are already stored in the database can be identified from the top rank entries. For our computations we have used both the SwissProt and PIR databases.

2.9. Sequence analysis of tryptic peptides

The tryptic peptides eluted from the polyacrylamide matrices were separated on a C18 Ultrasphere reverse-phase column (250 mm × 2 mm) with a 5%–60% linear gradient of acetonitrile containing 0.1% trifluoroacetic acid in 25 min, at a flow rate of 0.3 ml/min. Elution was monitored at 218 nm with a Spectroflow 783 detector, and peaks were manually collected. The N-terminal sequence analysis was performed on a gas-phase sequencer (470 A, Applied Biosystems), and amino acid analysis was carried out in a 420 A amino acid analyzer, with an on-line 130 A PTC analyzer (Applied Biosystems).

2.10. Statistical analysis

The GST subunits content data obtained in three different experiments with respect to diet manipulations (four individuals for each nutritional condition) were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet test of mean comparison with normal fed mice values as control, using a statistics analysis software (InStat, Graph Pad Software). P values lower than 0.05 were judged as significant.

3. Results

3.1. Identification of glutathione S-transferases

The SDS-PAGE analysis of liver cytosolic proteins demonstrated that protein depletion modifies the relative content of several polypeptides (Fig. 1A). In addition to GAPDH [3], the polypeptides of apparent molecular mass 27 000 Da (P27) and 25 000 Da (P25)

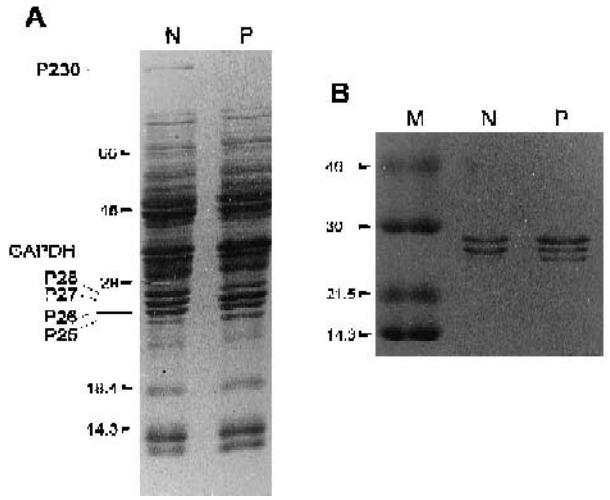

Fig. 1. Effect of protein depletion on cytosolic proteins and glutathione *S*-transferases. Mice were either fed with a normal (N) diet or exposed to protein depletion for 5 days (P).
A. Cytosolic proteins (25 μg) were analyzed by SDS-PAGE. Lines indicate the main changes observed in protein pattern. Molecular weight of marker proteins (kDa) are indicated by arrowheads.
B. Liver cytosolic GSTs were purified by *S*-hexyl-GSH–agarose affinity chromatography. The products obtained were analyzed by SDS-PAGE (3 μg of protein per track). M = molecular weight markers (kDa). A figure representative of 5 experiments is shown.

increased their relative content, while those of 230 000 Da (P230), 28 000 Da (P28) and 26 000 Da (P26) decreased. Thereafter, it was found that the N-terminal amino acid sequence of P27 is similar to that of Yb1, the most abundant mouse liver mu-class glutathione-*S*-transferase [18] (Fig. 2). Then, the liver cytosolic glutathione *S*-transferases (GSTs) from normal and protein depleted mice were isolated by affinity chromatography in *S*-hexylglutathione–agarose. Independently of the dietary protein condition tested, this procedure yielded a fraction composed by three different subunits of 27 000, 26 000 and 25 000 Da (Fig. 1B) containing 90–94% of the cytosolic GST activity. According to reports of several groups [18–22], these polypeptides should be mu-class Yb1, alpha-class Yc (Ya3) and pi-class Yf GST subunits, respectively. The composition of the affinity purified GST fraction was further analyzed by reverse-phase HPLC as Ostlund Farrants et al. [14]. Fig. 3 shows RP-HPLC profiles of liver GSTs obtained from control (A) and protein depleted (B) mice. Five peaks between 44% and 48.5% of acetonitrile were eluted and subjected to SDS-PAGE analysis. In every nutritional condition assayed, large peaks 2, 4 and 5 (94% of the area under the RP-HPLC profile) comprised P27, P26 and P25 subunits respectively; while the small peak 3 (4% of area) was composed by a P27, P26 and P25 mixture (Fig. 3C). Peak 1 accounted for the remaining 2% and was composed by 67 000-, 32 000- and 13 000-Da polypeptides.

For further identification, SDS-PAGE slices of P27, P26 and P25 bands were in-gel trypsin digested, and the resulting peptides subjected to mass spectrometric analysis as indicated in Section 2. A reliable approach to know the identity of a protein is to compare the mass of the tryptic peptides obtained with that of the theoretical peptides generated from a protein sequence data base [17]. After the experimental and theoretical molecular weight of the tryptic peptides were compared, the top rank entries were: for P27, mouse mu-class Yb1 GST [18]; for P26, mouse alpha-class Yc GST [23]; and for P25, mouse pi-class Yf GST [24,25]. Table 1 shows the molecular weights of the peptides found for the three proteins analyzed and their corresponding theoretical ones. The identification of P27 agreed with the N-terminal sequence analysis (Fig. 2). Since P26 matched in a minor grade with alpha-class Ya1 and Ya2 GSTs [23], its identity was further investigated. Thus P26 tryptic peptides were separated by RP-HPLC, analyzed by mass spectrometry and partially sequenced

A

P27	X M I L G Y X N V R G L
mouse Yb1	P M I L G Y W N V R G L T H P
mouse Yb2	P M T L G Y W D I R G L A H A

B

P26	(2162.4 Da) [a]	X X X X L M F **Q** Q V P M V
mouse Yc	(2160.5 Da) [b]	S D G S L M F **Q** Q V P M V E I D G M K
mouse Ya1/Ya2	(2087.4 Da) [b]	D G N L M F **D** Q V P M V E I D G M K

Fig. 2. Partial amino acid sequence of P27 and P26.
A. N-terminal aminoacid sequence of P27. The amino terminal of mouse class-mu Yb1 already reported [18] is also shown.
B. Comparison of the primary structure obtained from P26 with mouse Yc, Ya1 and Ya2 GSTs. Sequences of tryptic peptides of P26 were aligned with reported sequences of Yc, Ya1 and Ya2 [23].
[a] Measured fragment mass. [b] Theoretical fragment mass.
X denotes nonidentified amino acids. Experimental details are given in Section 2.

at their N-termini. The rationale of this experiment was that, although Yc, Ya1 and Ya2 are highly homologous [23], they can be identified by the amino acid sequence of determined fragments. Thus the Gln52 of the 45–63 (2162.4 Da) theoretical Yc tryptic fragment is replaced by Asp in the 46–63 (2087.4 Da) Ya1 and Ya2 homologue. Fig. 2 shows that the sequence of nine amino acids at N-terminal of the 2163.0-Da fragment yielded by P26 was identical to that of the corresponding Yc fragment.

The presence of other minor GST subunits was searched by a modification of the GSH-gradient elution from *S*-hexylglutathione–agarose chromatography described by Hayes et al. [18]. Fig. 4X shows the protein and activity profiles of gradient eluted GSTs from normal liver. The pools obtained (A–F) were subjected either to RP-HPLC or SDS-PAGE followed by trypsin digestion of bands and mass spectrometric analysis of the resulting fragments. The results indicated that Yf was present in pool A; Yc in pools B and C; and Yb1 in pools E and F, all of them highly purified (Fig. 4Y). In contrast, fraction D comprised Yc and Yb1 subunits (not shown). Similar results were obtained for all the dietary conditions tested in the present work.

3.2. Estimation of GST subunits amounts in normal, protein depleted and re-fed mice

The affinity chromatography isolated GSTs were submitted to RP-HPLC and the peak areas of the resulting subunits were compared with those in calibration curves obtained as follows: four amounts (2.5–15-μg range) of Yb1, Yc and Yf subunits were run in RP-HPLC (Fig. 4Y). A linear relationship between peak area and protein mass was found. As has been previously reported, each subunit displayed a different regression line slope [19]. Fig. 5 shows the total liver GST subunit contents in normal, five days protein depleted, and re-fed for 24- and 48-h mice. Protein depletion caused a significant change in GST

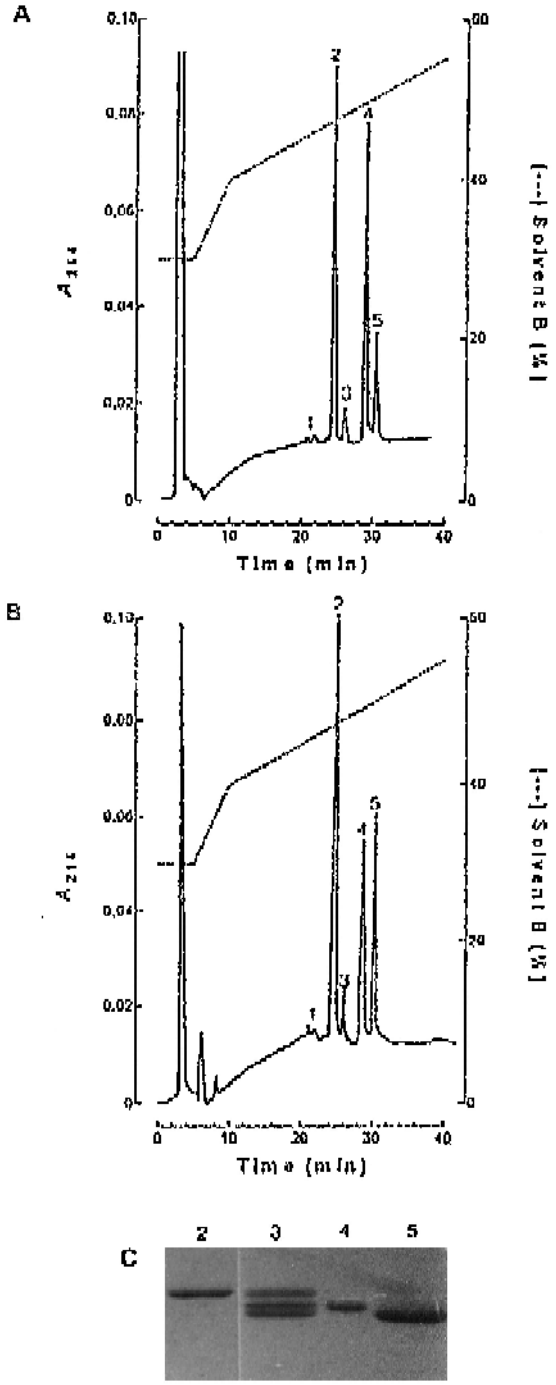

Fig. 3. Reverse-phase HPLC analysis of affinity isolated GSTs. Samples containing 25 μg protein were injected on a 250 mm × 4.6 mm Bio Rad C18 column and developed with a 30–55% (v/v) acetonitrile gradient in aq. 0.1% TFA. The eluate was monitored at 214 nm. Pump A delivered aq. 0.1% TFA and pump B 0.1% TFA (v/v) in 100% acetonitrile. Elution profiles: (A) GSTs from normal fed mice; (B) GSTs from protein depleted mice. Panel C shows the SDS-PAGE patterns of peaks 2–5 yielded by normal fed condition. Independent groups (four livers each) of normal fed and protein depleted mice were analyzed. Figure is representative of three different experiments in which each analysis was performed in triplicate.

Table 1
Summary of data obtained from mass spectrometry

P27 [a]	Yb1 [b]	Fragment [c]	P26 [a]	Yc [b]	Fragment [c]	P25 [a]	Yf [b]	Fragment [c]
732.3	727.9	(193–198)	823.2	819.9	(204–210)	1293.7	1292.5	(19–29)
790.4	792.9	(11–17)	853.7	857.0	(187–194)	1349.0	1351.6	(1–11)
954.6	956.1	(136–143)	901.8	901.0	(131–137)	1576.3	1577.7	(103–115)
972.6	976.1	(144–151)	933.5	932.0	(196–203)	1827.6	1824.9	(85–100)
1012.5	1015.2	(69–77)	1063.3	1060.2	(165–203)	1858.2	1855.9	(55–70)
1038.3	1039.2	(202–210)	1246.8	1245.3	(141–151)	1934.0	1937.1	(190–208)
1117.2	1119.2	(173–181)	1353.0	1359.5	(1–12)	2139.2	2135.4	(120–140)
1298.7	1303.3	(32–42)	1466.0	1462.6	(20–32)			
1346.5	1351.3	(83–93)	1792.6	1794.1	(69–83)			
1401.3	1406.5	(96–107)	2162.4	2160.5	(45–63)			
1475.3	1479.5	(82–93)	3026.1	3022.4	(155–181)			
1603.6	1609.6	(18–30)	3328.6	3330.8	(89–116)			
1898.1	1899.1	(52–68)						
1991.8	1994.2	(152–167)						

SDS-PAGE slices of P27, P26 and P25 were in-gel digested with trypsin [16] and the resulting peptides were subjected to mass spectrometric analysis. The experimental peptide masses were compared with those of the theoretical fragments generated from a protein sequence database with a mass limit tolerance of 6 Da, using a previously developed computer program [17]. Data are representative of at least three experiments.

[a] Measured fragment mass (Da).

[b] Theoretical fragment mass (Da).

[c] Amino acid position.

subunits proportions. While Yb1 and Yf content increased by $21.59 \pm 3.55\%$ and $78.46 \pm 5.51\%$, respectively, Yc content decreased $31.20 \pm 0.48\%$. Fig. 5 also shows that Yb1 and Yc contents practically recovered their normal values during the second day of refeeding. However, after two days of refeeding, Yf subunit remained significantly higher ($63.38 \pm 8.44\%$). It may be noted that changes in subunit contents similar to those presented in Fig. 5 were observed by densitometric analysis of cytosolic P27,

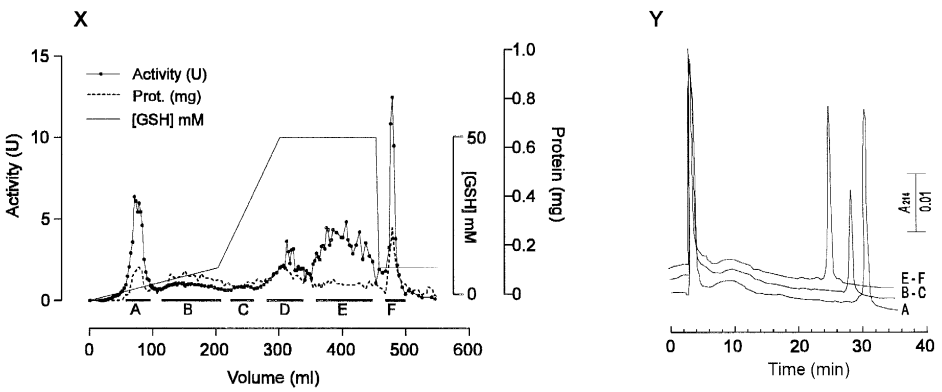

Fig. 4. Gradient elution of mouse GSTs from *S*-hexylglutathione–agarose and RP-HPLC profiles of the eluted fractions.
X. Normal fed cytosols (from four pooled mouse livers), were applied to a 1.6 cm × 7 cm *S*-hexylglutathione–agarose column. The matrix was washed and elution was carried in four stages as indicated in Section 2. Fractions of 2.6 ml were collected and tested for GST activity and protein content. Then they were pooled as indicated by horizontal bars (A–F).
Y. Pools A to F were subjected to RP-HPLC as described in Section 2 and Fig. 3. Samples of 5.90 μg (pools B, C, E and F) and 3.75 μg (pool A) were analyzed.

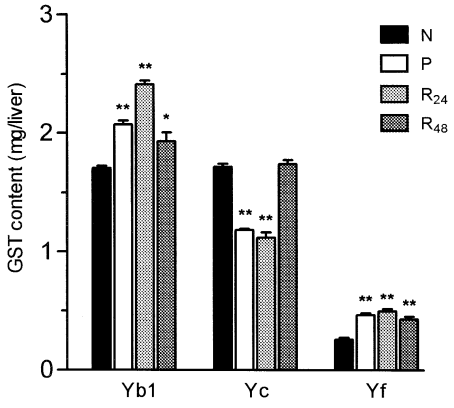

Fig. 5. Estimation of GST subunits contents during dietary manipulations. GSTs were affinity-isolated from liver cytosols (prepared from four livers) and subsequently submitted to RP-HPLC as described in Section 2 and Fig. 4. Contents were then assessed by comparison of the peak areas with those given by known amounts of subunits as detailed in Section 3. N = normal diet throughout; P = 5 days of protein depletion; R24, R48 = 24 and 48 h of refeeding, respectively, with normal diet after 5 days of protein depletion. Values represent the mean of three independent experiments and are expressed as percentage of the total area under the profile. Error bars represent S.E.M. The symbols above the bars indicate that the values are significantly different compared with normal control (N); * ($P < 0.05$); ** ($P < 0.01$).

P26 and P25 bands in SDS-PAGEs as those depicted in Fig. 1A and B (not shown).

3.3. Quantitation of GSTs mass and activity in normal, protein depleted and re-fed mice

In parallel with the estimations of subunits levels, the total GSTs content and activity were assessed (Table 2). Both the GSTs mass and activity per liver did not change with depletion and appeared to slightly increase after refeeding. Related to cytosolic protein mass, both GSTs content and activity increased with depletion and started to decrease during the second day of refeeding. On the other hand, the activity per enzyme mass (specific activity) was slightly higher than control during depletion and refeeding. In addition, from the percentages of GSTs it is possible to estimate the cytosolic protein contents. As previously described [2], the cytosolic protein decreases by 30.2% with depletion and is recovered with refeeding.

4. Discussion

In the present work, three liver cytosolic polypeptides whose content is regulated by dietary protein intake were identified as GST subunits. Their molecular weights, affinity chromatography and RP-HPLC profiles, peptide-mass fingerprints and amino acid sequences were identical to those described for mu-class Yb1, alpha-class Yc and pi-class Yf mouse GST subunits [18–22]. It was also found that, in every dietary protein condition tested, Yb1, Yc (also named Ya3) and Yf subunits account for more than 90% of the *S*-hexyl-GSH affinity purified liver cytosolic proteins. Similar recoveries have been reported for BALB/C [21] and CD-1 [19] strains. Moreover, in normal fed mice, the observed Yb1, Yc and Yf proportions were similar to those described for CD-1, DBA/2, C3H/He and C57BL6 strains [19,21]. In contrast with results indicating that certain chemical treatments of mice cause the appearance of liver GST

Table 2
Quantitation of GSTs mass and activity in normal, protein depleted and refed mice

Measurement	Dietary condition			
	N	P	R24	R48
GSTs:				
Content (mg/liver)	3.68 ± 0.04	3.71 ± 0.04	4.02 ± 0.03	4.09 ± 0.05
Activity (units/liver)	166.52 ± 9.82	179.90 ± 3.78	211.12 ± 1.18	213.00 ± 2.77
Specific activity (units/mg protein)	45.20 ± 3.11	48.60 ± 1.50	52.51 ± 0.76	52.08 ± 1.31
Activity (units/mg cytosolic protein)	2.24 ± 0.24	3.46 ± 0.07	3.57 ± 0.02	3.07 ± 0.04
% of cytosolic protein	4.95 ± 0.16	7.15 ± 0.33	6.80 ± 0.27	5.90 ± 0.32

Values are expressed in mean ± S.E.M. N = normal diet throughout; P = 5 days of protein depletion; R24, R48 = 24 and 48 h of refeeding, respectively, with normal diet after 5 days of protein depletion.

forms different from Yb1, Yc and Yf [18,19], it was found that protein depletion changes the proportions of constitutive GST homodimers, but does not induce the appearance of others. Thus the diminution in Yc caused by depletion is accompanied by a rise in Yb1 and Yf. Refeeding for two days practically restored the normal levels of Yb1 and Yc while Yf content remained high. The behaviour of Yc is consistent with data showing that rat alpha GST decreases with protein depletion and is completely recovered by refeeding [26]. Although Yc subunit appears to be unresponsive to drugs [27], protein depletion caused a marked decrease in its content. In contrast, the behaviour of Yb1 differs from that of rat mu GST which decreases with depletion and increases very slowly with refeeding [26]. It may be noted that substantial induction of Yb1 is also observed in mouse liver following treatment with BHA, PB, trans-stilbene oxide, cafestol palmitate and bisethylxanthogen [28].

Dietary protein depletion causes a decrease in synthesis and an increase in breakdown of liver proteins [1,2]. Then, the observed increased amounts of Yb1 and Yf, and the maintenance of total GSTs content, indicate that during protein depletion the GST subunits levels are controlled by mechanisms different from the majority of cytosolic proteins. In this way, we have previously found that, like GAPDH, the synthesis rate of P27 (now identified as Yb1 GST) increases with protein depletion and decreases during refeeding [3]. The Yf content could be regulated in a similar way as Yb1 during the same treatment. On the other hand, refeeding increases the synthesis and decreases the degradation of liver and kidney proteins [1,2,29]. Then, during refeeding, both the Yc recovery and the high level of Yf could be caused by an increased synthesis and/or a decreased breakdown. We are further investigating these possibilities.

The observed changes in both GST activity and mass per mg of cytosolic protein (Table 2) are different from the decrease and fast recovery of activity found in protein depleted and re-fed C57-Black female mice, respectively [10]. An explanation for these discrepancies could reside in the different mouse strains studied. However, it may be noted that GST mass related to cytosolic protein has not been estimated in C57 strain. Thus this study brought new information about the effect of dietary protein on the mouse GST mass, i.e. that both GST activity and content per liver do not fall during protein depletion. Again, since overall protein breakdown is increased [1,2], this fact could be assessed as striking. Thus the conclusion that particular mechanisms control GSTs levels is reinforced.

Although changes in the proportion of GST subunits have been found in response to several stimuli, their physiological meaning requires further study. The GSTs are known as enzymes that catalyze the nucleophilic attack of the sulfur atom of glutathione on electrophilic group in a second substrate. High amounts of GSTs are found in most forms of life investigated. It is considered that they are involved in the intracellular detoxification of several noxious chemical substances including carcinogens. The GSTs are either homo- or heterodimers coded in a multigenic family. Their genes could be induced by various xenobiotics and are expressed in a tissue-specific manner [8]. It has been emphasized that GST induction is part of an adaptative response mechanism to chemical stress that is widely distributed in nature [8]. Moreover, there is evidence suggesting that, besides providing protection against chemicals of foreign origin, GST are involved in protection against oxidative stress [8]. The pi-class Yf GST subunit has been described as a preneoplastic marker because of its conspicuous expression in rat liver pre-neoplastic foci [30]. McLellan and Hayes [21] found high levels of Yf subunit in male livers of three mouse strains and related this finding with the fact that spontaneous hepatomas occur with a much higher frequency in inbred male mice than in female. Hatayama et al. [31], postulated an hormonal regulation of Yf content because testosterone administration induced Yf in female mouse liver and male mouse castration decreased significantly the Yf liver content. Analyzing the links between GSTs and cancer, Tsuchida and Sato [9] suggest that pi-class GST may be a useful marker not only for various cancers, but also for high-risk precancerous lesions. It has been also described that alpha-class GST content decrease is characteristic in human liver and kidney cancer [32–34]. We have previously found that GAPDH content and synthesis rate increased during protein depletion [3]. High levels of GAPDH have also been detected during hepatic and renal carcinogenesis [4,5] and the

nutritional cycling of protein depletion–refeeding of rats has been associated with the liver cell predisposition for transformation [6]. Therefore, the study of the function and regulation of GST subunits and GAPDH by dietary protein level could contribute to a better understanding of carcinogenesis.

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